

| L Number | Hits | Search Text               | DB                                     | Time stamp       |
|----------|------|---------------------------|--|------------------|
| 1        | 286  | opsin                     | USPAT;<br>US-PGPUB;<br>EPO;<br>DERWENT | 2003/09/29 13:22 |
| 2        | 130  | clon? and opsin           | USPAT;<br>US-PGPUB;<br>EPO;<br>DERWENT | 2003/09/29 13:22 |
| 4        | 11   | rh2 and opsin             | USPAT;<br>US-PGPUB;<br>EPO;<br>DERWENT | 2003/09/29 13:23 |
| 3        | 2    | rh1 and (clon? and opsin) | USPAT;<br>US-PGPUB;<br>EPO;<br>DERWENT | 2003/09/29 13:23 |
| 5        | 11   | rh2 and opsin             | USPAT;<br>US-PGPUB;<br>EPO;<br>DERWENT | 2003/09/29 13:24 |

09/818143

File 5:Biosis Previews(R) 1969-2003/Sep W2  
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| Set | Items | Description  |
|-----|-------|--|
| S1  | 5090  | OPSIN  |
| S2  | 147   | CLON? AND S1                                       |
| S3  | 21    | RECEPTOR AND S2                                    |
| S4  | 17    | AU='WALKER MICHAEL G' OR AU='WALKER MICHAEL GREER' |
| S5  | 0     | S1 AND S4  |
| S6  | 1     | S4 AND RECEPTOR                                    |
| S7  | 17    | E3-E6  |
| S8  | 0     | S1 AND S7  |
| S9  | 0     | S7 AND RECEPTOR                                    |
| S10 | 12    | AU='KLINGER T' OR AU='KLINGER TOD M'               |
| S11 | 0     | S1 AND S10   |
| S12 | 0     | S10 AND RECEPTOR                                   |
| S13 | 8     | S1 AND CONSENSUS                                   |
| S14 | 25    | S1 AND RH1   |
| S15 | 16    | S1 AND RH2   |
| S16 | 0     | S14 AND CONSENSUS                                  |
| S17 | 0     | S15 AND CONSENSUS                                  |
| S18 | 2     | CLON? AND S14                                      |
| S19 | 4     | CLON? AND S15                                      |

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3/7/1

DIALOG(R)File 5:Biosis Previews(R)  
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14240386 BIOSIS NO.: 200300234415

Not all butterfly eyes are created equal: Rhodopsin absorption spectra, molecular identification, and localization of ultraviolet-, blue-, and green-sensitive rhodopsin-encoding mRNAs in the retina of *Vanessa cardui*.

AUTHOR: Briscoe Adriana D(a); Bernard Gary D; Szeto Allan S; Nagy Lisa M; White Richard H

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JOURNAL: Journal of Comparative Neurology 458 (4):p334-349 April 14 2003  
2003

MEDIUM: print

ISSN: 0021-9967

DOCUMENT TYPE: Article

RECORD TYPE: Abstract

LANGUAGE: English

ABSTRACT: Surveys of spectral sensitivities, visual pigment spectra, and opsins gene sequences have indicated that all butterfly eyes contain ultraviolet-, blue-, and green-sensitive rhodopsins. Some species also contain a fourth or fifth type, related in amino acid sequence to green-sensitive insect rhodopsins, but red shifted in absorbance. By combining electron microscopy, epimicrospectrophotometry, and polymerase chain reaction cloning, we found that the compound eye of *Vanessa cardui* has the typical ultrastructural features of the butterfly retina but contains only the three common insect rhodopsins. We estimated lambda-max values and relative densities of the rhodopsins in the *Vanessa* retina (0.72, P530; 0.12, P470; and 0.15, P360) from microspectrophotometric measurements and calculations based on a computational model of reflectance spectra. We isolated three opsins-encoding cDNA fragments that were identified with P530, P470, and P360 by homology to the well-characterized insect rhodopsin families. The retinal mosaic was mapped by opsins mRNA in situ hybridization and found to contain three kinds of ommatidia with respect to their patterns of short wavelength rhodopsin expression. In some ommatidia, P360 or P470 was expressed in R1 and R2 opposed receptor cells; in others, one cell expressed P360, whereas its complement expressed P470. P530 was expressed in the other seven cells of all ommatidia. P470-expressing cells were abundant in the ventral retina but nearly absent dorsally. Our results indicated that there are major differences between the color vision systems of nymphalid and papilionid butterflies: the nymphalid

Vanessa has a simpler, trichromatic, system than do the tetrachromatic papilionids that have been studied.

3/7/2

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13856167 BIOSIS NO.: 200200484988

\*\*\*Opsin\*\*\*-G11-mediated signaling pathway for photic entrainment of the chicken pineal circadian clock.

AUTHOR: Kasahara Takaoki; Okano Toshiyuki; Haga Tatsuya; Fukada Yoshitaka  
(a)

AUTHOR ADDRESS: (a)Department of Biophysics and Biochemistry, Graduate School of Science, University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo, 113-0033\*\*Japan E-Mail: sfukada@mail.ecc.u-tokyo.ac.jp

JOURNAL: Journal of Neuroscience 22 (17):p7321-7325 September 1, 2002

MEDIUM: print

ISSN: 0270-6474

DOCUMENT TYPE: Article

RECORD TYPE: Abstract

LANGUAGE: English

ABSTRACT: Light is a major environmental signal for entrainment of the circadian clock, but little is known about the intracellular phototransduction pathway triggered by light activation of the photoreceptive molecule(s) responsible for the phase shift of the clock in vertebrates. The chicken pineal gland and retina contain the autonomous circadian oscillators together with the photic entrainment pathway, and hence they represent useful experimental models for the clock system. Here we show the expression of G11alpha, an alpha subunit of heterotrimeric G-protein, in both tissues by cDNA \*\*\*cloning\*\*\*, Northern blot, and Western blot analyses. G11alpha immunoreactivity was colocalized with pinopsin in the chicken pineal cells and also with rhodopsin in the outer segments of retinal photoreceptor cells, suggesting functional coupling of G11alpha with opsins in the clock-containing photosensitive tissues. The physical interaction was examined by coimmunoprecipitation experiments, the results of which provided evidence for light- and GTP-dependent coupling between rhodopsin and G11alpha. To examine whether activation of endogenous G11 leads to a phase shift of the oscillator, Gq/11-coupled m1-type muscarinic acetylcholine \*\*\*receptor\*\*\* (mAChR) was ectopically expressed in the cultured pineal cells. Subsequent treatment of the cells with carbamylcholine (CCh), an agonist of mAChR, induced phase-dependent phase shifts of the melatonin rhythm in a manner very similar to the effect of light. In contrast, CCh treatment induced no measurable effect on the rhythm of nontransfected (control) cells or cells expressing Gi/o-coupled m2-type mAChR, indicating selectivity of the G-protein activation. Together, our results demonstrate the existence of a G11-mediated \*\*\*opsin\*\*\*-signaling pathway contributing to the photic entrainment of the circadian clock.

3/7/3

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13371830 BIOSIS NO.: 200200000651

Species-specific differences in expression of G-protein-coupled \*\*\*receptor\*\*\* kinase (GRK) 7 and GRK1 in mammalian cone photoreceptor cells: Implications for cone cell phototransduction.

AUTHOR: Weiss Ellen R; Ducceschi Melissa H; Horner Thierry J; Li Aimin; Craft Cheryl M; Osawa Shoji(a)

AUTHOR ADDRESS: (a)Department of Cell and Developmental Biology, The University of North Carolina at Chapel Hill, CB No. 7090, 108 Taylor Hall, Chapel Hill, NC, 27599-7090\*\*USA E-Mail: shoosawa@med.unc.edu

JOURNAL: Journal of Neuroscience 21 (23):p9175-9184 December 1, 2001

MEDIUM: print

ISSN: 0270-6474

DOCUMENT TYPE: Article

RECORD TYPE: Abstract

LANGUAGE: English

**ABSTRACT:** Desensitization plays an important role in the rapid termination of G-protein signaling pathways. This process, which involves phosphorylation by a G-protein-coupled **receptor** kinase (GRK) followed by arrestin binding, has been studied extensively in the rod photoreceptor cell of the mammalian retina. In contrast, less is known regarding desensitization in cone photoreceptor cells, which occurs more rapidly than in rod cells. Recently, our laboratory has **cloned** a novel GRK family member, GRK7, from the retina of a cone-dominant mammal, the 13-lined ground squirrel. Here we report the **cloning** of GRK7 from rod-dominant pig and human retinas, suggesting that this kinase plays a role in human visual signaling. Because GRK1 (rhodopsin kinase), the GRK that mediates rhodopsin desensitization in the rod cell, is reportedly expressed in both rods and cones, a detailed comparison of the localization of the two kinases is a necessary step toward determining their potential roles in cone visual signaling. Immunocytochemical analysis using antibodies selective for these two GRKs unexpectedly demonstrated species-specific differences in GRK7 and GRK1 expression in cones. In pigs and dogs, cones express only GRK7, whereas in mice and rats, we detected only GRK1 in cones. These results suggest that either GRK7 or GRK1 may participate in cone **opsin** desensitization, depending on the expression pattern of the kinases in different species. In contrast, GRK7 and GRK1 are coexpressed in monkey and human cones, suggesting that coordinate regulation of desensitization by both kinases may occur in primates.

3/7/4

DIALOG(R)File 5:Biosis Préviews(R)  
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12890367 BIOSIS NO.: 200100097516

Distribution of photoreceptor classes and retinal specialization in relation to visually guided behavior in *Manduca sexta*.

AUTHOR: White R H(a)

AUTHOR ADDRESS: (a)University of Massachusetts Boston, Boston, MA\*\*USA

JOURNAL: Society for Neuroscience Abstracts 26 (1-2):pAbstract No-4468  
2000

MEDIUM: print

CONFERENCE/MEETING: 30th Annual Meeting of the Society of Neuroscience New Orleans, LA, USA November 04-09, 2000

SPONSOR: Society for Neuroscience

ISSN: 0190-5295

RECORD TYPE: Abstract

LANGUAGE: English

SUMMARY LANGUAGE: English

**ABSTRACT:** The *Manduca* retina consists of about 27,000 retinulae each made up of 9 receptors. Antisera were raised against the 3 rhodopsins (P357, P450, P520) previously characterized and **cloned** as retinal cDNA. Immunocytochemistry was used to determine which retinular cells express each photopigment, and to map the distribution of photoreceptor types across the retina. The 2 **receptor** cells with distal rhabdoms express P357 (UV receptors). The 6 cells with more proximal rhabdoms variably express P520 (green receptors) or P450 (blue receptors), with the latter generally arranged as opposite pairs. The rhodopsin of the small, most proximal cell has not been identified. Immunocytochemistry also suggests that there may be another **opsin** expressed in the retina that has not been identified. The **receptor** types are distributed unequally across the retina in relation to functional requirements. In particular, behavioral experiments with artificial colored "flowers" support the earlier conclusion that flower visitation by these nocturnal foragers is mediated mainly by the blue receptors. The *Manduca* retina also contains a large (approx. 1000 retinulae), distinctive area at the dorsal rim of the eye, with retinulae that show morphological features of polarized light detectors, as in other insect eyes, and a distinctive distribution of the **receptor** classes.

3/7/5

DIALOG(R)File 5:Biosis Previews(R)  
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12813608 BIOSIS NO.: 200100020757

Expression of a recombinant human RGR **opsin** in Lentivirus-transduced cultured cells.

AUTHOR: Yang Mao; Wang Xiao-guang; Stout J Timothy; Chen Pu; Hjelmeland Leonard M; Appukuttan Binoy; Fong Henry K W(a)

AUTHOR ADDRESS: (a)Doheny Eye Institute, 1355 San Pablo Street, Los Angeles, CA, 90033: hfong@hsc.usc.edu\*\*USA

JOURNAL: Molecular Vision 6 (32 CITED DEC. 12, 2000):p237-242 Nov. 18, 2000

MEDIUM: online

ISSN: 1090-0535

DOCUMENT TYPE: Article

RECORD TYPE: Abstract

LANGUAGE: English

SUMMARY LANGUAGE: English

ABSTRACT: Purpose: Our goals were to produce a functional recombinant RPE retinal G protein-coupled **receptor** (RGR) **opsin** for biochemical studies and to test the efficiency of a lentiviral vector for transgene expression of human RGR. Methods: A human RGR cDNA was **cloned** into a replication-defective lentiviral vector, and recombinant hRGR-Lentivirus was prepared for transduction of the ARPE-19, a human retinal pigment epithelium (RPE) cell line, and COS-7 cells. Recombinant RGR expression was detected by Western blot analysis, and functionality of the protein was tested by a (3H)all-trans-retinal binding assay. Results: RGR protein was detected in each cell type after transduction with recombinant virus and was not observed in untreated cells. RGR expression in ARPE-19 cells increased steadily for up to 10 days after transduction and was stable for at least 6 months. The transduced ARPE-19 cells produced approx100-fold higher amounts of RGR protein than the transduced COS-7 cells. When cell membranes from the ARPE-19 cells were incubated with (3H)all-trans-retinal, the chromophore bound specifically to the expressed protein. Uptake of (3H)all-trans-retinal into the ARPE-19 cells was followed by specific binding of radiolabeled retinoid to RGR. Conclusions: Using a Lentivirus-derived gene delivery system, we were able to express high amounts of human RGR protein in the ARPE-19 human RPE cell line. The transduced ARPE-19 cells remain able to process all-trans-retinal, and the expressed protein is capable of binding to the all-trans-retinal chromophore. The Lentivirus-based expression of functional RGR can be used to study RGR in cultured cells and to test in vivo transduction of quiescent RPE cells.

3/7/6

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12317238 BIOSIS NO.: 200000038266

Heterologous gene expression in a membrane-protein-specific system.

AUTHOR: Turner George J(a); Reusch Regina; Winter-Vann Ann M; Martinez Lynell; Betlach Mary C

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JOURNAL: Protein Expression and Purification 17 (2):p312-323 Nov., 1999

ISSN: 1046-5928

DOCUMENT TYPE: Article

RECORD TYPE: Abstract

LANGUAGE: English

SUMMARY LANGUAGE: English

ABSTRACT: We have constructed an expression system for heterologous proteins which uses the molecular machinery responsible for the high level production of bacteriorhodopsin in Halobacterium salinarum. **Cloning** vectors were assembled that fused sequences of the bacterio-**opsin** gene (bop) to coding sequences of heterologous genes and generated DNA fragments with **cloning** sites that permitted transfer of fused genes into H. salinarum expression vectors. Gene

fusions include: (i) carboxyl-terminal-tagged bacterio-**opsin**; (ii) a carboxyl-terminal fusion with the catalytic subunit of the Escherichia coli aspartate transcarbamylase; (iii) the human muscarinic **receptor**, subtype M1; (iv) the human serotonin **receptor**, type 5HT2c; and (v) the yeast alpha mating factor **receptor**, Ste2. Characterization of the expression of these fusions revealed that the bop gene coding region contains previously undescribed molecular determinants which are critical for high level expression. For example, introduction of immunogenic and purification tag sequences into the C-terminal coding region significantly decreased bop gene mRNA and protein accumulation. The bacteriorhodopsin-aspartate transcarbamylase fusion protein was expressed at 7 mg per liter of culture, demonstrating that E. coli codon usage bias did not limit the system's potential for high level expression. The work presented describes initial efforts in the development of a novel heterologous protein expression system, which may have unique advantages for producing multiple milligram quantities of membrane-associated proteins.

3/7/7

DIALOG(R)File 5:Biosis Previews(R)  
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11803547 BIOSIS NO.: 199900049656

Structure and developmental expression of the mouse RGR **opsin** gene.

AUTHOR: Tao Li; Shen Daiwei; Pandey Sujay; Hao Wenshan; Rich Kathryn A;  
Fong Henry K W(a)

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CA, 90033

JOURNAL: Molecular Vision 4 (25 CITED DEC. 9, 1998):pNO PAGINATION Dec. 2,  
1998

ISSN: 1090-0535

DOCUMENT TYPE: Article

RECORD TYPE: Abstract

LANGUAGE: English

ABSTRACT: Purpose: The aim of this study is to isolate and characterize cDNA **clones** and the genes that encode mouse RPE retinal G protein-coupled **receptor** (RGR) and to analyze expression of the RGR gene in the developing mouse retina. The conserved amino acid sequences of RGR from various mammals can be compared to the amino acid sequence motif of G protein-coupled receptors. Methods: Mouse RGR cDNA and gene **clones** were isolated from a retina cDNA library and 129SV genomic DNA library, respectively. The expression of RGR in the developing C57BL/6J mouse retina was analyzed by immunohistochemical staining with a polyclonal antipeptide antibody. Results: The deduced amino acid sequence of mouse RGR is 78% and 81% identical to that of bovine and human RGR, respectively. The mouse RGR gene is split into seven exons and extends about 11 kb. Two predominant mRNA transcripts, 1.9 and 1.7 kb in length, and a third, relatively faint, 5.5-kb transcript were detected in mouse eye by hybridization to a RGR cDNA probe. Frozen sections of C57BL/6J mouse retina at various stages of development were incubated with a mouse RGR antipeptide antibody. RGR immunoreactivity was first seen at postnatal day 2 (P2) in centrally located RPE cells. From day P6 to P12, there was an increase in the number and intensity of immunoreactive RPE cells in the central and mid-peripheral regions of the retina, while the most peripheral RPE cells were still negative. By day P16, the length of the RPE monolayer was immunoreactive, and staining of the central RPE cells was markedly more intense than at younger ages. Conclusions: Mouse and human RGR are highly conserved. A gradient of RGR expression in RPE extends from the central to the peripheral retina during development. In reference to the appearance of melanin-positive differentiated RPE cells, the induction of RGR expression is a relatively late event in the maturation of the retina.

3/7/8

DIALOG(R)File 5:Biosis Previews(R)  
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11802317 BIOSIS NO.: 199900048426

The **cloning** of GRK7, a candidate cone **opsin** kinase, from cone- and rod-dominant mammalian retinas.  
AUTHOR: Weiss Ellen R; Raman Dayanidhi; Shirakawa Satoko; Ducceschi Melissa H; Bertram Paul T; Wong Fulton; Kraft Timothy W; Osawa Shoji(a)  
AUTHOR ADDRESS: (a)Dep. Cell Biol. and Anat., Univ. North Carolina at Chapel Hill, CB 7090, 108 Taylor Hall, Chapel\*\*USA  
JOURNAL: Molecular Vision 4 (27 CITED DEC. 9, 1998):pNO PAGINATION Dec. 8, 1998  
ISSN: 1090-0535  
DOCUMENT TYPE: Article  
RECORD TYPE: Abstract  
LANGUAGE: English

ABSTRACT: Purpose: Desensitization in the rod cell of the mammalian retina is initiated when light-activated rhodopsin is phosphorylated by the G protein-coupled **receptor** kinase (GRK), GRK1, often referred to as rhodopsin kinase. A distinct kinase that specifically phosphorylates cone opsins in a similar manner has not been identified in mammals. To determine the existence of a cone **opsin** kinase, RNA from the retinas of cone- and rod-dominant mammals was analyzed by PCR. Methods: RNA prepared from the retinas of two cone-dominant mammals, the thirteen-lined ground squirrel and the eastern chipmunk, and a rod-dominant mammal, the pig, was used to **clone** a new GRK family member by RT-PCR. The tissue distribution and localization of the kinase in retina were determined by Northern blot hybridization and in situ hybridization. The protein encoded by this cDNA was expressed in human embryonic kidney-293 (HEK-293) cells and compared with bovine GRK1 for its ability to phosphorylate bovine rhodopsin and to undergo autophosphorylation. Results: The cDNA **cloned** from ground squirrel contains an open reading frame encoding a 548 amino-acid protein. Sequence analysis indicates that this protein is orthologous to GRK7 recently **cloned** from *O. latipes*, the medaka fish. Partial cDNA fragments of GRK7 were also **cloned** from RNA prepared from eastern chipmunk and pig retinas. In situ hybridization demonstrated widespread labeling in the photoreceptor layer of the ground squirrel retina, consistent with expression in cones. Recombinant ground squirrel GRK7 phosphorylates bovine rhodopsin in a light-dependent manner and can be autophosphorylated, similar to bovine GRK1. Conclusions: These results indicate that cone- and rod-dominant mammals both express GRK7. The presence of this kinase in cones in the ground squirrel and its ability to phosphorylate rhodopsin suggests that it could function in cone cells as a cone **opsin** kinase.

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DIALOG(R)File 5:Biosis Previews(R)  
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11593945 BIOSIS NO.: 199800374650  
Identification of G protein-coupled receptors in insect cells.  
BOOK TITLE: Annals of the New York Academy of Sciences; Trends in comparative endocrinology and neurobiology: From molecular to integrative biology  
AUTHOR: vanden Broeck Jozef; Poels Jeroen; Simonet Gert; Dickens Louis; De Loof Arnold  
BOOK AUTHOR/EDITOR: Vaudry H; Tonon M-C; Roubos E W; de Loof A: Eds  
AUTHOR ADDRESS: Lab. Developmental Physiol. Mol. Biol., Zoological Inst., Dep. Biol. K.U. Leuven, Naamsestraat 59, B\*\*Belgium  
JOURNAL: Annals of the New York Academy of Sciences 839p123-128 May 15, 1998  
BOOK PUBLISHER: New York Academy of Sciences, 2 East 63rd Street, New York, New York 10021, USA  
CONFERENCE/MEETING: 18th Conference of European Comparative Endocrinologists: From Molecular to Integrative Biology Rouen, France September 10-14, 1996  
SPONSOR: European Society for Comparative Endocrinology  
ISSN: 0077-8923 ISBN: 1-57331-110-3 (cloth); 1-57331-111-1 (paper)  
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LANGUAGE: English

3/7/10  
DIALOG(R)File 5:Biosis Previews(R)  
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11026620 BIOSIS NO.: 199799647765  
The rat D-4 dopamine **receptor** couples to cone transducin (G-alpha-r2) to inhibit forskolin-stimulated cAMP accumulation.  
AUTHOR: Yamaguchi Ikuyo; Harmon Steven K; Todd Richard D; O'Malley Karen L (a)  
AUTHOR ADDRESS: (a)Dep. Anat. Neurol., Washington Univ. Sch. Med., Box 8108, 660 S. Euclid Ave., St. Louis, MO 6311\*\*USA  
JOURNAL: Journal of Biological Chemistry 272 (26):p16599-16602 1997  
ISSN: 0021-9258  
RECORD TYPE: Abstract  
LANGUAGE: English

ABSTRACT: Based on its expression pattern and pharmacology, the D-4 dopamine **receptor** may play a role in schizophrenia. Thus it is of interest to know what signaling pathways are utilized by this **receptor**. Previously, we showed that activation of D-4 receptors in a mouse mesencephalic neuronal cell line (MN9D) inhibited forskolin-stimulated cAMP accumulation in a pertussis toxin-sensitive (Ptx-sensitive) fashion. Of the known Ptx-sensitive G-protein alpha subunits, MN9D-expressed G-alpha-i2, G-alpha-oA, and G-alpha-b; however, none of these coupled to the D-4 **receptor**. Using a low stringency polymerase chain reaction **cloning** method, we found an additional Ptx-sensitive G-protein cone transducin (G-alpha-t2) expressed in the MN9D cells. We also found that G-alpha-t2 mRNA is highly expressed in rat mesencephalic tissue. To test the hypothesis that the D-4 **receptor** couples to G-alpha-t2, we cotransfected MN9D cells with the D-4 **receptor** and a mutagenized Ptx-resistant G-alpha-t2 subunit (mG-alpha-t2). Application of the dopaminergic agonist quinpirole to cotransfected cells inhibited forskolin-stimulated cAMP accumulation in the presence or absence of Ptx. To our knowledge, this is the first report demonstrating that the D-4 dopamine **receptor** functionally couples to a specific G-protein and that a non-**opsin**-like **receptor** can couple with a transducin subunit.

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DIALOG(R)File 5:Biosis Previews(R)  
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10889764 BIOSIS NO.: 199799510909  
Molecular **cloning** of a rhodopsin gene from salamander rods.  
AUTHOR: Chen Ning; Ma Jian-Xing; Corson D Wesley; Hazard E Starr; Crouch Rosalie K  
AUTHOR ADDRESS: Dep. Ophthalmology, Med. Univ. South Carolina, 171 Ashley Avenue, Charleston, SC 29425\*\*USA  
JOURNAL: Investigative Ophthalmology & Visual Science 37 (9):p1907-1913 1996  
ISSN: 0146-0404  
RECORD TYPE: Abstract  
LANGUAGE: English

ABSTRACT: Purpose. Salamander photoreceptor cells have been used widely as models in vision research. However, the salamander **opsin** genes had not been **cloned**. The purpose of this study was to **clone** a salamander rhodopsin and to determine its primary structure and cell type-specific expression. Methods. Using salamander retina RNA as a template and Xenopus rhodopsin-specific oligonucleotides as primers, reverse transcription and polymerase chain reaction (RT-PCR) were used to amplify and **clone** a rhodopsin cDNA fragment. This fragment was used as a probe to isolate a full-length cDNA of the rhodopsin from a cDNA library of salamander retina. The dideoxynucleotide chain termination method was used to determine the nucleotide sequence. Single rod and cone cells were isolated by micromanipulation, and the absorbance spectra of the rod outer segments were measured with a photon-counting microspectrophotometer. Individual rod and cone cells were lysed for RT-PCR and Southern blot analysis to detect cell-specific expression of this gene. Results. A 1.2 kb rhodopsin cDNA containing the full-length



coding region of rhodopsin has been **cloned** and sequenced from the larval tiger salamander, *Ambystoma tigrinum*. This cDNA encodes 354 amino acids that, by hydropathy profile, could form seven transmembrane domains characteristic of other rhodopsins. Sequence identity was found with other amphibian rhodopsins at the nucleic acid (82% to 83%) and the amino acid (88% to 89%) levels. Key amino acids critical for structure and function of rhodopsin have been retained. The mRNA of this rhodopsin was identified in red rod cells (lambda-max 506 nm). No expression of the gene was detected in cone cells. Conclusions. The **cloned** rhodopsin is a newly isolated member of the G protein-coupled **receptor** superfamily. This protein is expressed in rods but not in cones.

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DIALOG(R)File 5:Biosis Previews(R)  
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10649166 BIOSIS NO.: 199699270311

Molecular **cloning** and localization of rhodopsin kinase in a mammalian pineal.

AUTHOR: Zhao X(a); Huang J; Fariss R N; Milam A H; Palczewski K  
AUTHOR ADDRESS: (a)Dep. Ophthalmol., Univ. Washington, Seattle, WA  
98195-6485\*\*USA

JOURNAL: Society for Neuroscience Abstracts 22 (1-3):p1814 1996  
CONFERENCE/MEETING: 26th Annual Meeting of the Society for Neuroscience  
Washington, D.C., USA November 16-21, 1996  
ISSN: 0190-5295  
RECORD TYPE: Citation  
LANGUAGE: English

3/7/13

DIALOG(R)File 5:Biosis Previews(R)  
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10482717 BIOSIS NO.: 199699103862

**Cloning** and expression analysis of *Aedes aegypti* **opsin**:  
Adaptation of an in situ hybridization protocol for mosquitoes.

AUTHOR: Graf R(a); Godknecht A; Nakano M; Li X; Ackermann U; Helbling P  
AUTHOR ADDRESS: (a)Dep. Chirurgie DL36, Sternwartstrasse 14,  
Universitatsspital Zurich, 8091 Zurich\*\*Switzerland  
JOURNAL: Insect Molecular Biology 5 (3):p173-180 1996

ISSN: 0962-1075  
DOCUMENT TYPE: Article  
RECORD TYPE: Abstract  
LANGUAGE: English

ABSTRACT: **Opsin** is a G protein coupled photoreceptor that activates a signal transduction cascade in the ommatidia. Its primary and secondary structure is conserved both in insects and vertebrates as exemplified by the *Drosophila* opsins. Through serendipitous **cloning** of a PCR fragment, we have identified an **opsin** cDNA. The latter was used to **clone** full length cDNAs from a mosquito head library. The main purpose of **cloning** was to have a positive control probe to establish an in situ hybridization protocol for less abundant probes. **Opsin**-mRNA is localized specifically to the visual **receptor** cells in the ommatida. No other cells in the brain or the remainder of the body are positive. This is confirmed by Northern blot analysis. The sequence of the **receptor**, of which we have found two different transcripts, confirms its typical topology, including the seven transmembrane spanning regions and the intracellular carboxy terminus that has potential phosphorylation sites. Our in situ hybridization protocol combines several procedures: the most important points are: (a) the immediate processing of sections after cutting, and (b) the sections are never allowed to dry out once the procedure was started. Our protocol has a much higher sensitivity, using approximately 50x lower concentrations of probe compared to published protocols. In addition to the detection of **opsin**-mRNA, it has been successfully applied to the detection of the low abundant insulin **receptor** homologue. Furthermore, *Aedes aegypti* probes were visualizing a similar tissue specificity when applied to the malaria mosquito *Anopheles albimanus*.

3/7/14

DIALOG(R)File 5:Biosis Previews(R)  
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09819348 BIOSIS NO.: 199598274266

Alternative splicing in human retinal mRNA transcripts of an **opsin**-related protein.

AUTHOR: Jiang Meisheng; Shen Daiwei; Tao Li; Pandey Sujay; Heller Keith; Fong Henry K W(a)

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JOURNAL: Experimental Eye Research 60 (4):p401-406 1995

ISSN: 0014-4835

DOCUMENT TYPE: Article

RECORD TYPE: Abstract

LANGUAGE: English

ABSTRACT: An **opsin**-related gene encodes a putative RPE-retinal G-protein-coupled **receptor** (RGR) that is most homologous to the visual pigments and invertebrate retinochrome. A splice variant of human RGR mRNA can be demonstrated by the sequence of isolated cDNA **clones** and by the amplification and analysis of human retinal mRNA. The shortened transcript contains a deletion of 114 nucleotides that correspond exactly to the sequence of exon 6 in the human rgr gene. The predicted RGR variant lacks the putative sixth transmembrane domain and has a calculated molecular weight of 27726. Variable amounts of a 28-kDa protein were found in the retinas of some individuals by immunoblot assay. Since a similar shortened RGR transcript was not detected in bovine retina or RPE, the RGR variant is not essential for vertebrate vision. Analysis of the structure of the rgr gene and of the sequences of cDNA **clones** indicates that the truncated mRNA may be produced through alternative splicing of pre-mRNA from which a cassette exon is removed and the predicted RGR variant is radically altered in primary structure.

3/7/15

DIALOG(R)File 5:Biosis Previews(R)  
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09764242 BIOSIS NO.: 199598219160

Pineal **opsin**: A nonvisual **opsin** expressed in chick pineal.

AUTHOR: Max Marianna(a); McKinnon Peter J; Seidenman Kenneth J; Barrett R Keith; Applebury Meredith L; Takahashi Joseph S; Margolskee Robert F

AUTHOR ADDRESS: (a)Roche Inst. Molecular Biol., Roche Res. Center, Hoffman-LaRoche, Nutley, NJ 07110\*USA

JOURNAL: Science (Washington D C) 267 (5203):p1502-1506 1995

ISSN: 0036-8075

DOCUMENT TYPE: Article

RECORD TYPE: Abstract

LANGUAGE: English

ABSTRACT: Pineal **opsin** (P-**opsin**), an **opsin** from chick that is highly expressed in pineal but is not detectable in retina, was **cloned** by the polymerase chain reaction. It is likely that the P-**opsin** lineage diverged from the retinal opsins early in **opsin** evolution. The amino acid sequence of P-**opsin** is 42 to 46 percent identical to that of the retinal opsins. P-**opsin** is a seven-membrane spanning, G protein-linked **receptor** with a Schiff-base lysine in the seventh membrane span and a Schiff-base counterion in the third membrane span. The primary sequence of P-**opsin** suggests that it will be maximally sensitive to approx 500-nanometer light and produce a slow and prolonged phototransduction response consistent with the nonvisual function of pineal photoreception.

3/7/16

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09089018 BIOSIS NO.: 199497097388

An **opsin** homologue in the retina and pigment epithelium.

AUTHOR: Jiang Meisheng; Pandey Sujay; Fong Henry K W(a)

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90033\*USA

JOURNAL: Investigative Ophthalmology & Visual Science 34 (13):p3669-3678  
1993

ISSN: 0146-0404

DOCUMENT TYPE: Article

RECORD TYPE: Abstract

LANGUAGE: English

ABSTRACT: Purpose. The aim of this project was to investigate the retinal pigment epithelium (RPE) at the molecular level by identification of novel RPE-specific cDNAs that may encode proteins of signal transduction pathways or other proteins that are expressed preferentially in the RPE. Methods. A bovine RPE cDNA library was constructed in bacteriophage lambda-g10 using RPE-enriched poly(A)+ RNA. The library was screened by differential hybridization to bovine RPE and kidney cDNA probes. Results. A member of the heptahelical **receptor** family was identified in bovine RPE by molecular **cloning**. Its deduced amino acid sequence predicts a protein that has 291 amino acid residues and resembles most closely the family of visual pigments. A lysine residue, analogous to the retinaldehyde attachment site in rhodopsin, is conserved in the seventh hydrophobic segment of the novel sequence. Messenger RNA encoding the putative G protein-coupled **receptor** was detected by in situ hybridization in the RPE, inner nuclear layer, and specific cells of the ganglion cell layer. Immunohistochemical staining of bovine retina showed that the **receptor** protein is localized in Muller cells, as well as in the RPE. Conclusions. A novel heptahelical **receptor** defines a distant evolutionary branch of the visual pigment tree. The selective localization of this putative **receptor**, its abundance in RPE and retina, and its homology to the visual pigments suggest that the function of this **receptor** is important in a visual process involving the RPE and Muller cells.

3/7/17

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06274519 BIOSIS NO.: 000086108702

ECTOPIC EXPRESSION OF A MINOR DROSOPHILA **OPSIN** IN THE MAJOR

PHOTORECEPTOR CELL CLASS DISTINGUISHING THE ROLE OF PRIMARY

**RECEPTOR** AND CELLULAR CONTEXT

AUTHOR: ZUKER C S; MISMER D; HARDY R; RUBIN G M

AUTHOR ADDRESS: DEP. BIOCHEM., UNIV. CALIFORNIA, BERKELEY, CALIF. 94720.

JOURNAL: CELL 53 (3). 1988. 475-482. 1988

FULL JOURNAL NAME: Cell

CODEN: CELLB

RECORD TYPE: Abstract

LANGUAGE: ENGLISH

ABSTRACT: We have used P-element-mediated transformation to introduce the **cloned** Rh1 rhodopsin gene into the germ line of Drosophila and fully rescue the visual phenotype of mutant ninaE flies. A transcriptional fusion between the ninaE promoter and the structural gene for a minor **opsin** (Rh2) that is not normally expressed in the R1-R6 photoreceptor cells was used to demonstrate that Rh2 rhodopsin can photoactivate the R1-R6 transduction cascade, but with different spectral sensitivity. In addition, we show that two mutants that specifically affect the R1-R6 cells, ninaA and rdgB, do not directly affect expression of the ninaE gene.

3/7/18

DIALOG(R)File 5:Biosis Previews(R)

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05601110 BIOSIS NO.: 000083074250

COMPLEMENTARY DNA FOR THE HUMAN BETA-2-ADRENERGIC \*\*\*RECEPTOR\*\*\* A PROTEIN  
WITH MULTIPLE MEMBRANE-SPANNING DOMAINS AND ENCODED BY A GENE WHOSE  
CHROMOSOMAL LOCATION IS SHARED WITH THAT OF THE \*\*\*RECEPTOR\*\*\* FOR  
PLATELET-DERIVED GROWTH FACTOR

AUTHOR: KOBILKA B K; DIXON R A F; FRIELLE T; DOHLMAN H G; BOLANOWSKI M A;  
SIGAL I S; YANG-FENG T L; FRANCKE U; CARON M G; LEFKOWITZ R J  
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27710.

JOURNAL: PROC NATL ACAD SCI U S A 84 (1). 1987. 46-50. 1987

FULL JOURNAL NAME: Proceedings of the National Academy of Sciences of the  
United States of America

CODEN: PNASA

RECORD TYPE: Abstract

LANGUAGE: ENGLISH

ABSTRACT: We have isolated and sequence a cDNA encoding the human  
.beta.2-adrenergic \*\*\*receptor\*\*\*. The deduced amino acid sequence (413  
residues) is that of a protein containing seven clusters of hydrophobic  
amino acids suggestive of membrane-spanning domains. While the protein is  
87% identical overall with the previously \*\*\*clones\*\*\* hamster  
.beta.2-adrenergic \*\*\*receptor\*\*\*, the most highly conserved regions are  
the putative transmembrane helices (95% identical) and cytoplasmic loops  
(93% identical), suggestin that these regions of the molecular harbor  
important functional domains. Several of the transmembrane helices also  
share lesser degrees of identity with comparable regions of select  
members of the \*\*\*opsin\*\*\* family of visual pigments. We have localized  
the gene for the .beta.-adrenergic \*\*\*receptor\*\*\* to q31-q32 on  
chromosome 5. This is the same position recently determined for the gene  
encoding the \*\*\*receptor\*\*\* for platelet-derived growth factor and is  
adjacent to that for the FMS protooncogene, which encodes the  
\*\*\*receptor\*\*\* for the macrophage colony-stimulating factor.

3/7/19

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04221639 BIOSIS NO.: 000077047684

THE SUB CELLULAR LOCALIZATION OF RAT PHOTO \*\*\*RECEPTOR\*\*\* SPECIFIC ANTIGENS

AUTHOR: FEKETE D M; BARNSTABLE C J

AUTHOR ADDRESS: DEP. ANAT., HARVARD MED. SCH., BOSTON, MA 02115, USA.

JOURNAL: J NEUROCYTOL 12 (5). 1983. 785-804. 1983

FULL JOURNAL NAME: Journal of Neurocytology

CODEN: JNCYA

RECORD TYPE: Abstract

LANGUAGE: ENGLISH

ABSTRACT: The subcellular localization of 3 photoreceptor antigens (RET-P1,  
rhodopsin and RET-P2) has been studied by EM immunocytochemistry of rat  
retinas. Localization was also examined by determining the amount of  
RET-P1 and RET-P2 antigen in various subcellular fractions. RET-P1 and  
RET-P2 antigens were further characterized by immunoblotting of crude  
retina membrane proteins which had been separated by 1-dimensional gel  
electrophoresis. RET-P1 antigen has been detected with a monoclonal  
antibody that reacts with the perikarya, inner segments, and outer  
segments of adult rat photoreceptors by peroxidase immunolabeling of  
fixed tissue sections. Analysis at the EM level has shown that RET-P1  
antigen is located on the external face of the inner and outer segment  
plasma membrane. A monoclonal antibody against purified bovine rhodopsin  
(RHO-C7) labels the outer segments of rat retinas by peroxidase  
immunocytochemistry. Ultrastructural antibody localization indicates that  
this particular determinant of rhodopsin is exposed on the external face  
of the plasma membrane of outer segments and may also be expressed on the  
surface of the inner segments. RET-P2 antibody labels only the outer  
segments of adult rat photoreceptors by peroxidase immunocytochemistry.  
The light microscopic labeling of RET-P2 antibody in the presence, but  
not in the absence, of detergent suggests that it is an intracellular  
antigen. The results of both ultrastructural labeling and biochemical  
fractionation are consistent with the localization of RET-P2 antigen on  
the internal face of the plasma membrane and/or the cytoplasmic face of  
the disc membranes. RET-P2 antigen was a protein (or glycoprotein) of

apparent MW of 38,000 +- 3000.

3/7/20

DIALOG(R)File 5:Biosis Previews(R)  
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03867633 BIOSIS NO.: 000075045706  
ORGANIZATION OF RHOD \*\*\*OPSIN\*\*\* AND A HIGH MOLECULAR WEIGHT GLYCO PROTEIN  
IN ROD PHOTO \*\*\*RECEPTOR\*\*\* DISC MEMBRANES USING MONO \*\*\*CLONAL\*\*\*  
ANTIBODIES  
AUTHOR: MACKENZIE D; MOLDAV R S  
AUTHOR ADDRESS: DEP. BIOCHEMISTRY, UNIV. BRITISH COLUMBIA, VANCOUVER,  
BRITISH COLUMBIA V6T 1W5 CANADA.  
JOURNAL: J BIOL CHEM 257 (12). 1982. 7100-7105. 1982  
FULL JOURNAL NAME: Journal of Biological Chemistry  
CODEN: JBCHA  
RECORD TYPE: Abstract  
LANGUAGE: ENGLISH

ABSTRACT: Four monoclonal antibodies obtained from the fusion of mouse myeloma cells with lymphocytes of mice immunized with bovine rod outer segment disc membranes were shown to bind to the surface of sealed discs. Radioimmune labeling of rod outer segment membrane proteins separated by sodium dodecyl sulfate gel electrophoresis indicated that 2 monoclonal antibodies (3D6 and 4B4) were against rhodopsin. Limited proteolysis of rod outer segment membranes with trypsin and Streptomyces griseus protease indicated that the 3D6 antibody bound to the trypsin-sensitive region close to the carboxyl-terminal end of rhodopsin. The 4B4 antibody bound at a trypsin-insensitive, but S. griseus protease-sensitive internal region of rhodopsin accessible on the cytoplasmic surface of discs. Two other monoclonal antibodies (3D12 and 4B2) were found to bind to different regions of the Mr [molecular ratio] = 220,000 concanavalin A binding glycoprotein of rod outer segment disc membranes. These antibodies also bound to a Mr = 140,000 fragment which does not contain the concanavalin A binding site. Immunoferritin-labeling studies for transmission electron microscopy confirm the location of the 3D6 and 4B2 antigens on the cytoplasmic or interdisc surface of disc membranes.

3/7/21

DIALOG(R)File 5:Biosis Previews(R)  
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03743401 BIOSIS NO.: 000024071474  
PHOTO \*\*\*RECEPTOR\*\*\* SPECIFIC MONO \*\*\*CLONAL\*\*\* ANTIBODIES  
AUTHOR: WONG F; LEONARD R B; KREMER N E; DENNEY R M  
AUTHOR ADDRESS: MARINE BIOMED. INST., UTMB, GALVESTON, TX.  
JOURNAL: ANNUAL SPRING MEETING OF THE ASSOCIATION FOR RESEARCH IN VISION AND OPHTHALMOLOGY INCORPORATED, SARASOTA, FLA., USA, MAY 2-7, 1982. INVEST OPHTHALMOL VISUAL SCI 22 (3 SUPPL.). 1982. 205. 1982  
CODEN: IOVSD  
DOCUMENT TYPE: Meeting  
RECORD TYPE: Citation  
LANGUAGE: ENGLISH

? t s6/3/1

6/3/1

DIALOG(R)File 5:Biosis Previews(R)  
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13717924 BIOSIS NO.: 200200346745  
Z39Ig is co-expressed with activated macrophage genes.  
AUTHOR: \*\*\*Walker Michael G\*\*\* (a)  
AUTHOR ADDRESS: (a)Incyte Genomics, 3160 Porter Drive, Palo Alto, CA, 94304  
\*\*USA E-Mail: mwalker@stanfordalumni.org  
JOURNAL: Biochimica et Biophysica Acta 1574 (3):p387-390 12 April, 2002  
MEDIUM: print  
ISSN: 0006-3002

DOCUMENT TYPE: Article  
RECORD TYPE: Abstract  
LANGUAGE: English

? t s13/7/1-8

13/7/1  
DIALOG(R) File 5: Biosis Previews(R)  
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14171642 BIOSIS NO.: 200300165671  
Efficient and Allele-specific Hammerhead Ribozymes Targeted Against Human P23H Rhodopsin mRNA and Production of Human P23H Rhodopsin Transgenic Mice: Step Two Towards ADRP Gene Therapy.  
AUTHOR: Fritz J J(a); Lewin A S; Hauswirth W W  
AUTHOR ADDRESS: (a) Molecular Genetics and Microbiology, Ophthalmology, Powell Gene Therapy Center, Powell Gene Therapy Center, University of Florida, Gainesville, FL, USA\*\*USA  
JOURNAL: ARVO Annual Meeting Abstract Search and Program Planner 2002p Abstract No 4588 2002  
MEDIUM: cd-rom  
CONFERENCE/MEETING: Annual Meeting of the Association For Research in Vision and Ophthalmology Fort Lauderdale, Florida, USA May 05-10, 2002  
RECORD TYPE: Abstract  
LANGUAGE: English

ABSTRACT: Purpose: In the US, P23H is the most prevalent of the known rhodopsin point mutations responsible for autosomal dominant retinitis pigmentosa (ADRP). Our goals are to develop efficient and allele specific hammerhead ribozymes for use in a human gene therapy protocol for the treatment of P23H rhodopsin-linked ADRP and, to generate a transgenic mouse model of ADRP in which to assay the therapeutic promise of these ribozymes. Methods: Several related hammerhead ribozymes were designed to target an RNA oligonucleotide substrate containing the sequence of human P23H rod ~~rhodopsin~~ mRNA associated with ADRP. Modifications were made in both hybridizing arms, in the functionally conserved stem-loop, as well as in the ~~consensus~~ catalytic core to enhance the ribozyme's kinetic properties. Cleavage time course analyses were performed using short synthetic RNA substrates containing either the human wild-type or P23H rod ~~rhodopsin~~ mRNA sequences to determine the selectivity and catalytic efficiency of the ribozymes. Hammerheads that selectively cleaved the human P23H rod ~~rhodopsin~~ RNA substrate and not the normal sequence were subjected to further in vitro kinetic analysis and were cloned into a AAV vector for the production of recombinant virus particles. Additionally, transgenic mice carrying the human P23H rod ~~rhodopsin~~ gene have been produced by pronuclear microinjection. Results: Two related ribozymes, the 654 and 654 U10 hammerheads, were highly selective for and efficient at cleaving the human P23H rhodopsin substrate and each have been cloned into a an rAAV vector. Transgenic mice carrying the human P23H rhodopsin transgene have been successfully produced and are currently undergoing genetic and phenotypic analysis to determine their suitability as a murine model of ADRP. Conclusion: Hammerhead ribozymes can be optimized for discriminating between the human P23H and wild-type rod ~~rhodopsin~~ RNA species that differ by a single nucleotide. Kinetic data suggest that the 654 and 654 U10 hammerheads should provide a therapeutic effect by reducing the amount of aberrant P23H rhodopsin in rod photoreceptors.

13/7/2  
DIALOG(R) File 5: Biosis Previews(R)  
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11813288 BIOSIS NO.: 199900059397  
not really finished is crucial for development of the zebrafish outer retina and encodes a transcription factor highly homologous to human nuclear respiratory factor-1 and avian initiation binding repressor.  
AUTHOR: Becker Thomas S; Burgess Shawn M; Amsterdam Adam H; Allende Miguel L; Hopkins Nancy(a)  
AUTHOR ADDRESS: (a) Dep. Biol., Mass. Inst. Technol., Cambridge, MA 01239\*\*

USA

JOURNAL: Development (Cambridge) 125 (22):p4369-4378 Nov., 1998

ISSN: 0950-1991

DOCUMENT TYPE: Article

RECORD TYPE: Abstract

LANGUAGE: English

ABSTRACT: Not really finished (nrf), a larval-lethal mutation in zebrafish generated by retroviral insertion, causes specific retinal defects. Analysis of mutant retinæ reveals an extensive loss of photoreceptors and their precursors around the onset of visual function. These neurons undergo apoptosis during differentiation, affecting all classes of photoreceptors, suggesting an essential function of nrf for the development of all types of photoreceptors. In the mutant, some photoreceptors escape cell death, are functional and, as judged by **opsin** expression, belong to at least three classes of cones and one class of rods. The protein encoded by nrf is a close homologue of human Nuclear Respiratory Factor 1 and avian Initiation Binding Repressor, transcriptional regulators binding the upstream **consensus** sequence RCGCRYGCGY. At 24 hours of development, prior to neuronal differentiation, nrf is expressed ubiquitously throughout the developing retina and central nervous system. At 48 hours of development, expression of nrf is detected in the ganglion cell layer, in the neurons of the inner nuclear layer, and in the optic nerve and optic tracts, and, at 72 hours of development, is no longer detectable by in situ hybridization. Mutants contain no detectable nrf mRNA and die within 2 weeks postfertilization as larvae with reduced brain size. On the basis of its similarity with NRF-1 and IBR, nrf is likely growth factor receptors and other transcription factors. This demonstrates the power of insertional mutagenesis as a means for characterizing novel genes necessary for vertebrate retinal development.

13/7/3

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11617091 BIOSIS NO.: 199800398885

Molecular cloning of the salamander red and blue cone visual pigments.

AUTHOR: Xu Lin; Hazard E Starr III; Lockman D Kashelle; Crouch Rosalie K; Ma Jian-Xing(a)

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JOURNAL: Molecular Vision 4 (10 CITED JULY 28, 1998):pNO PAGINATION July 15, 1998

MEDIUM: online

ISSN: 1090-0535

DOCUMENT TYPE: Article

RECORD TYPE: Abstract

LANGUAGE: English

ABSTRACT: Purpose: Salamander retinas are known to contain at least three cone pigments and two rod pigments. The purpose of this study was to clone and characterize the visual pigments from salamander cones. Methods: cDNA fragments of cone pigments were amplified from a salamander retina cDNA library by PCR using a pair of primers with **consensus** for visual pigments. These fragments were cloned and used as probes for library-screening. The full-length cDNAs were isolated from the retinal library using the cloned PCR products as probes. DNA sequences were determined by the dideoxynucleotide chain termination method. Results: Two pigment cDNAs were cloned and sequenced from the salamander library. The global GenBank search showed that they do not match any existing sequences but have significant sequence similarity to visual pigments. One of the pigment cDNAs showed a high sequence homology with red cone pigments from other species and thus, was designated as a red cone **opsin**. The other pigment was designated as a blue cone **opsin** as it is most homologous to the chicken and goldfish blue cone pigments. Both cDNAs contain a full-length coding region encoding 365 amino acids in the red and 363 amino acids in the blue cone pigment. Hydrophathy analysis predicted that both pigments could form seven hydrophobic transmembrane helices. Both pigments retain the key amino acid residues

critical for maintaining the structure and function of opsins and have similar G-protein interaction sequences which differ from that of rod **opsin**. Phylogenetic analysis indicates that the red **opsin** belongs to the L group and the blue **opsin** belongs to the M1 group of visual pigments. Conclusions: The salamander red and blue cone pigments share high sequence homology with the cone pigments of other species.

13/7/4

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11428725 BIOSIS NO.: 199800210057

A novel subtype of G-protein-coupled receptor kinase, GRK7, in teleost cone photoreceptors.

AUTHOR: Hisatomi Osamu; Matsuda Shinji; Satoh Takunori; Kotaka Shuichi; Imanishi Yoshikazu; Tokunaga Fumio(a)

AUTHOR ADDRESS: (a)Dep. Earth Space Sci., Grad. Sch. Sci., Osaka Univ., Machikaneyama-cho 1-1, Toyonaka, Osaka 560-**Japan**

JOURNAL: FEBS Letters 424 (3):p159-164 March 13, 1998

ISSN: 0014-5793

DOCUMENT TYPE: Article

RECORD TYPE: Abstract

LANGUAGE: English

ABSTRACT: Two kinds of retinal cDNA fragments (OlGRK-R and -C) encoding the putative G-protein-coupled receptor kinases (GRKs) were isolated from medaka, *Oryzias latipes*. OlGRK-R appears to be closely related to the rhodopsin kinase (RK) found in the outer segments of mammalian photoreceptors, but the deduced amino acid sequence of OlGRK-C shows less than 50% identity to those of GRKs known to date, suggesting that OlGRK-C is a novel GRK subtype (GRK7). The mRNA of OlGRK-R is detectable in rods, and that of OlGRK-C is found in all four types of cone photoreceptor. The C-terminal of OlGRK-R has a **consensus** sequence for farnesylation, whereas, surprisingly, OlGRK-C has a **consensus** sequence for geranylgeranylation. Our result are consistent with the concept that lower vertebrates have rod- and cone-specific **opsin** kinases.

13/7/5

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09977516 BIOSIS NO.: 199598432434

Characterization and regulation of the protein binding to a cis-acting element, RET 1, in the rat **opsin** promoter.

AUTHOR: Yu Xiu; Barnstable Colin J(a)

AUTHOR ADDRESS: (a)Dep. Ophthalmology and Visual Sci., Yale Univ. Sch. Med., New Haven, CT-**USA**

JOURNAL: Journal of Molecular Neuroscience 5 (4):p259-271

ISSN: 0895-8696

DOCUMENT TYPE: Article

RECORD TYPE: Abstract

LANGUAGE: English

ABSTRACT: RET 1 is a binding site for retinal nuclear proteins located at -136 to -110 bp in the rat **opsin** promoter, as defined by DNase protection assays. A similar sequence is found in the upstream flanking regions of many other photoreceptor genes in mammals and other species, including *Drosophila*. A 7-base **consensus** sequence, CAATTAG, is found in these genes and has the binding activity of the longer RET 1 element. A 40-kDa protein that binds to RET 1 has been purified over 2 times 10<sup>5</sup>-fold to apparent homogeneity by affinity chromatography. The RET 1 binding activity is first detectable at E18 and increases during the first two postnatal weeks. At embryonic ages the retarded bands show an altered mobility and at early postnatal ages two bands are detected, with the adult band increasing and the embryonic band decreasing in intensity. Treatment of early postnatal retinas with bFGF increased the binding activity in nuclear extracts and caused a shift in migration of the retarded band to a position characteristic of the embryonic form of



the complex. The results support the hypothesis that RET 1-like elements play an important role in rod photoreceptor development.

13/7/6

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09313054 BIOSIS NO.: 199497321424

Structure and function in rhodopsin: The role of asparagine-linked glycosylation.

AUTHOR: Kaushal Shalesh; Ridge Kevin D; Khorana H Gobind(a)

AUTHOR ADDRESS: (a)Dep. Biol. Chem., Massachusetts Inst. Technol., 77  
Massachusetts Ave., Cambridge, MA 02139\*\*USA

JOURNAL: Proceedings of the National Academy of Sciences of the United  
States of America 91 (9):p4024-4028 1994

ISSN: 0027-8424

DOCUMENT TYPE: Article

RECORD TYPE: Abstract

LANGUAGE: English

ABSTRACT: Rhodopsin, the dim light photoreceptor of the rod cell, is an integral membrane protein that is glycosylated at Asn-2 and Asn-15. Here we report experiments on the role of the glycosylation in rhodopsin folding and function. Nonglycosylated rhodopsin was prepared by expression of a wild-type bovine rhodopsin gene in COS-1 cells in the presence of tunicamycin, an inhibitor of asparagine-linked glycosylation. The nonglycosylated rhodopsin folded correctly as shown by its normal palmitoylation, transport to the cell surface, and the formation of the characteristic rhodopsin chromophore (lambda-max, 500 nm) with 11-cis-retinal. However, the nonglycosylated rhodopsin showed strikingly low light-dependent activation of GT at concentration levels comparable with those of glycosylated rhodopsin. Amino acid replacements at positions 2 and 15 and the cognate tripeptide consensus sequence (Asn-2 fowardw Gln, Gly-3 fowardw Cys (Pro), Thr-4 fowardw Lys, Asn-15 fowardw Ala (Cys, Gln, Lys, Gln, Arg), Lys-16 fowardw Cys (Arg), Thr-17 Met (Val)) showed that the substitutions at Asn-2, Gly-3, and Thr-4 had no significant effect on the folding, cellular transport, and/or function of rhodopsin, whereas those at Asn-15 and Lys-16 caused poor folding and were defective in transport to the cell surface. Further, mutant pigments with amino acid replacements at Asn-15 and Thr-17 activated G-T very poorly. We conclude that Asn-15 glycosylation is important in signal transduction.

13/7/7

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09214601 BIOSIS NO.: 199497222971

A fourth gene in the bop gene cluster of Halobacterium halobium is co-regulated with the bop gene.

AUTHOR: Gropp Felix; Gropp Roswitha; Betlach Mary C(a)

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Francisco, San Francisco, CA 94143\*\*USA

JOURNAL: Systematic and Applied Microbiology 16 (4):p716-724 1994

ISSN: 0723-2020

DOCUMENT TYPE: Article

RECORD TYPE: Abstract

LANGUAGE: English

ABSTRACT: We have detected an additional gene in the bop gene cluster of the halophilic archaeon, Halobacterium halobium. This gene, denoted blp (bacterio-rhodopsin linked product) is located directly beyond the 3' terminus of the bat gene and is transcribed in the opposite orientation. blp transcription paralleled bop transcription under various growth conditions in the wild-type and in the majority of mutants tested, including Bop regulatory mutants. Moreover, blp transcription was induced by low oxygen tension in a similar manner to that previously observed for bop transcription. These data suggest that the blp and bop genes are co-regulated by low oxygen tension. Striking sequence similarities

observed up to 90 bp upstream of the blp and the bop genes may represent binding sites for regulatory factors mediating the oxygen response. Sequence identity between the -25 regions upstream of these two genes revealed a motif which is noncanonical with respect to the archeal \*\*\*consensus\*\*\* promoter and provides the best definition to date of the bop promoter.

13/7/8

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06953570 BIOSIS NO.: 000089075576

THE PRIMARY STRUCTURE OF A HALORHODOPSIN FROM NATRONOBACTERIUM-PHARAONIS-  
STRUCTURAL FUNCTIONAL AND EVOLUTIONARY IMPLICATIONS FOR BACTERIAL  
RHODOPSINS AND HALORHODOPSINS

AUTHOR: LANYI J K; DUSCHL A; HATFIELD G W; MAY K; OESTERHELT D  
AUTHOR ADDRESS: DEP. PHYSIOLOGY BIOPHYSICS, UNIV. CALIFORNIA, IRVINE,  
CALIF. 92717.

JOURNAL: J BIOL CHEM 265 (3). 1990. 1253-1260. 1990

FULL JOURNAL NAME: Journal of Biological Chemistry

CODEN: JBCHA

RECORD TYPE: Abstract

LANGUAGE: ENGLISH

ABSTRACT: We cloned and sequenced the gene coding for the polypeptide of a halorhodopsin in Natronobacterium pharaonis (named here pharanonis halorhodopsin). Peptide sequencing of cyanogen bromide fragments, and immunoreactions of the protein and synthetic peptides derived from the COOH-terminal gene sequence, confirmed that the open reading frame is the structural gene for the pharnis halorhodopsin polypeptide. The flanking DNA sequences, as well as those for other bacterial rhodopsins, were compared to previously proposed arachaeobacterial \*\*\*consensus\*\*\* sequences. In pair-wise comparisons of the open reading frame with DNA sequences for bacterio-\*\*\*opsin\*\*\* and halo-\*\*\*opsin\*\*\* from Halobacterium halobium, silent divergences (mutations/nucleotide at codon positions which do not result in amino acid changes) were calculated. These indicate very considerable evolutionary distance between each pair of genes. In spite of this, the three protein sequences show extensive similarities, indicating strong selective pressures. Conserved and converatively replaced amino acid residues in all three proteins identify general features essential for ion-motive bacterial rhodopsins, responsible for overall structure and chromophore properties. Comparison of the bacteriorhodopsin sequence with those of two halorhodopsins, on the other hand, identifies features involved in their specific (proton and chloride ion) transport functions.

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18/7/1

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14161432 BIOSIS NO.: 200300155461

Upstream Sequence of Retinal \*\*\*Opsin\*\*\* Genes Capitulates Transcriptional  
Control of Those Genes in Oncorhynchus mykiss.

AUTHOR: Dann S G(a); Allison W T(a); Hawryshyn C W(a)

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Canada

JOURNAL: ARVO Annual Meeting Abstract Search and Program Planner 2002p

Abstract No 3639 2002

MEDIUM: cd-rom

CONFERENCE/MEETING: Annual Meeting of the Association For Research in  
Vision and Ophthalmology Fort Lauderdale, Florida, USA May 05-10, 2002

RECORD TYPE: Abstract

LANGUAGE: English

ABSTRACT: Purpose: Salmonid fish such as rainbow trout (O. mykiss) experience loss of the ultraviolet sensitive photoreceptor (SWS1 cone) via apoptosis, and thus photosensitivity, during a metamorphosis-like event. This event, referred to as smoltification, is driven by release of

thyroid hormone (T3) and results in physiological and anatomical changes in the fish. Our goal is to determine if ~~rhodopsin~~ gene transcription is directly affected by T3 through its nuclear receptors (THR) or through other transcription factors (TF) whose expression is linked to T3. Methods: A simple method for attaining upstream sequence from known genes is genome walking. Briefly, genomic DNA from the organism of interest is digested with restriction endonucleases that produce blunt ends. Adapters of known sequence are ligated to blunt end DNA and nested PCR is performed with two forward primers matching the adapter and two reverse primers matching the 5' end of the gene of interest. Data from ~~cloned~~ and sequenced PCR products are subjected to TF search(<http://www.cbrc.jp/research/db/TFSEARCH.html>), which identifies sites of known TF-DNA binding. Results: 500 to 2000bp of upstream sequence have been generated from each of the retinal ~~opsin~~ genes identified in O. mykiss i.e. SWS1, SWS2, LWS, ~~Rh1~~, Rh2. TF binding sites have been identified in each of these gene's promoter regions, a summary of which will be presented in this poster. Of particular interest is a palindromic AP-1 binding site found in the upstream sequence of the SWS1 gene. Conclusion: Identifying the TFs involved in ~~opsin~~ gene expression is the first step in creating genomic and proteomic paradigms, which attempt to describe neurodegenerative events in the salmon retina. The AP-1 site upstream of the SWS1 ~~opsin~~ gene, for instance, binds activating protein - 1, a TF known to exert effects in cis with THR in the human malic enzyme and ChAT. In fact Schmidt et al. (1993) have shown that nuclear THR represses transcriptional activation by AP-1 in a T3-dependent fashion. AP-1 has also been shown to regulate expression of genes central to the apoptotic pathway, specifically Bcl-x, Bax, and Bcl-3. In order to understand mechanisms controlling apoptosis of SWS1 cones in salmon retina we are seeking the mechanisms controlling transcription of genes central to its cellular function. Similarly we are attempting to identify mechanisms that may confer proliferative events on adjacent photoreceptors in the salmon retina. This work supported by Fellowships from the Alzheimer Society of B.C./Canada (WTA) and NSERC (SGD), and by grants from NSERC (CWH).

18/7/2

DIALOG(R)File 5:Biosis Previews(R)  
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06274519 BIOSIS NO.: 000086108702  
ECTOPIC EXPRESSION OF A MINOR DROSOPHILA ~~OPSIN~~ IN THE MAJOR  
PHOTORECEPTOR CELL CLASS DISTINGUISHING THE ROLE OF PRIMARY RECEPTOR AND  
CELLULAR CONTEXT

AUTHOR: ZUKER C S; MISMER D; HARDY R; RUBIN G M  
AUTHOR ADDRESS: DEP. BIOCHEM., UNIV. CALIFORNIA, BERKELEY, CALIF. 94720.  
JOURNAL: CELL 53 (3). 1988. 475-482. 1988  
FULL JOURNAL NAME: Cell  
CODEN: CELLB  
RECORD TYPE: Abstract  
LANGUAGE: ENGLISH

ABSTRACT: We have used P-element-mediated transformation to introduce the ~~cloned~~ ~~Rh1~~ rhodopsin gene into the germ line of Drosophila and fully rescue the visual phenotype of mutant ninaE flies. A transcriptional fusion between the ninaE promoter and the structural gene for a minor ~~opsin~~ (Rh2) that is not normally expressed in the R1-R6 photoreceptor cells was used to demonstrate that Rh2 rhodopsin can photoactivate the R1-R6 transduction cascade, but with different spectral sensitivity. In addition, we show that two mutants that specifically affect the R1-R6 cells, ninaA and rdgB, do not directly affect expression of the ninaE gene.

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19/7/1

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14427409 BIOSIS NO.: 200300421438  
Identification of circadian brain photoreceptors mediating photic entrainment of behavioural rhythms in lizards.

AUTHOR: Pasqualetti Massimo; Bertolucci Cristiano; Ori Michela; Innocenti Augusto; Magnone Maria C; de Grip Willem J; Nardi Irma; Foa Augusto(a)  
AUTHOR ADDRESS: (a)Dipartimento di Biologia e Centro di Neuroscienze, Università di Ferrara, Via L. Borsari 46, I-44100, Ferrara, Italy\*\*Italy  
E-Mail: foa@unife.it  
JOURNAL: European Journal of Neuroscience 18 (2):p364-372 July 2003 2003  
MEDIUM: print  
ISSN: 0953-816X  
DOCUMENT TYPE: Article  
RECORD TYPE: Abstract  
LANGUAGE: English

ABSTRACT: We have shown previously that in ruin lizards (*Podarcis sicula*) the ablation of all known photoreceptive structures (lateral eyes, pineal and parietal eye) in the same individual animal does not prevent entrainment of their circadian locomotor rhythms to light. The present study was aimed at identifying the circadian brain photoreceptors mediating entrainment. For this purpose, we looked for *opsin* expression in the brain by means of immunocytochemistry. Using anti-cone-*opsin* antiserum CERN 874 we have localized photoreceptors in the periventricular area of hypothalamus, near the third cerebral ventricle. We also *cloned* a brain *opsin* cDNA that, on the basis of the deduced amino acid sequence, appears to belong to the *RH2* class of cone-opsins. We named the *cloned* cone-*opsin* Ps-*RH2*. To examine whether brain cone-opsins mediate photic entrainment of circadian locomotor rhythms, we performed post-transcriptional inactivation experiments by injecting an expression eukaryotic vector transcribing the antisense cone-*opsin* Ps-*RH2* mRNA in the third cerebral ventricle of pinealectomized-retinectomized lizards previously entrained to a light-dark (LD) cycle. Injections of the antisense construct abolished photic entrainment of circadian locomotor rhythms of pinealectomized-retinectomized lizards to the LD cycle for 6-9 days. CERN 874 completely failed to label cells within the periventricular area of hypothalamus of brains injected with antisense construct. Thus, abolishment of photic entrainment is due to inactivation of endogenous brain cone-opsins mRNA. The present results demonstrate for the first time in a vertebrate that brain cone-opsins are part of a true circadian brain photoreceptor participating in photic entrainment of behavioural rhythms.

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14161432 BIOSIS NO.: 200300155461  
Upstream Sequence of Retinal *Opsin* Genes Capitulates Transcriptional Control of Those Genes in *Oncorhynchus mykiss*.  
AUTHOR: Dann S G(a); Allison W T(a); Hawryshyn C W(a)  
AUTHOR ADDRESS: (a)Biology, University of Victoria, Victoria, BC, Canada\*\*  
Canada  
JOURNAL: ARVO Annual Meeting Abstract Search and Program Planner 2002p  
Abstract No 3639 2002  
MEDIUM: cd-rom  
CONFERENCE/MEETING: Annual Meeting of the Association For Research in Vision and Ophthalmology Fort Lauderdale, Florida, USA May 05-10, 2002  
RECORD TYPE: Abstract  
LANGUAGE: English

ABSTRACT: Purpose: Salmonid fish such as rainbow trout (*O. mykiss*) experience loss of the ultraviolet sensitive photoreceptor (SWS1 cone) via apoptosis, and thus photosensitivity, during a metamorphosis-like event. This event, referred to as smoltification, is driven by release of thyroid hormone (T3) and results in physiological and anatomical changes in the fish. Our goal is to determine if *opsin* gene transcription is directly affected by T3 through its nuclear receptors (THR) or through other transcription factors (TF) whose expression is linked to T3. Methods: A simple method for attaining upstream sequence from known genes is genome walking. Briefly, genomic DNA from the organism of interest is digested with restriction endonucleases that produce blunt ends. Adapters of known sequence are ligated to blunt end DNA and nested PCR is

performed with two forward primers matching the adapter and two reverse primers matching the 5' end of the gene of interest. Data from **cloned** and sequenced PCR products are subjected to TF search(<http://www.cbrc.jp/research/db/TFSEARCH.html>), which identifies sites of known TF-DNA binding. Results: 500 to 2000bp of upstream sequence have been generated from each of the retinal **opsin** genes identified in O. mykiss i.e. SWS1, SWS2, LWS, RH1, **RH2**. TF binding sites have been identified in each of these gene's promoter regions, a summary of which will be presented in this poster. Of particular interest is a palindromic AP-1 binding site found in the upstream sequence of the SWS1 gene. Conclusion: Identifying the TFs involved in **opsin** gene expression is the first step in creating genomic and proteomic paradigms, which attempt to describe neurodegenerative events in the salmon retina. The AP-1 site upstream of the SWS1 **opsin** gene, for instance, binds activating protein - 1, a TF known to exert effects in cis with THR in the human malic enzyme and ChAT. In fact Schmidt et al. (1993) have shown that nuclear THR represses transcriptional activation by AP-1 in a T3-dependent fashion. AP-1 has also been shown to regulate expression of genes central to the apoptotic pathway, specifically Bcl-x, Bax, and Bcl-3. In order to understand mechanisms controlling apoptosis of SWS1 cones in salmon retina we are seeking the mechanisms controlling transcription of genes central to its cellular function. Similarly we are attempting to identify mechanisms that may confer proliferative events on adjacent photoreceptors in the salmon retina. This work supported by Fellowships from the Alzheimer Society of B.C./Canada (WTA) and NSERC (SGD), and by grants from NSERC (CWH).

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09873178 BIOSIS NO.: 199598328096  
Paralogous origin of the rhodopsin-like **opsin** genes in lizards.  
AUTHOR: Kawamura Shoji; Yokoyama Shozo(a)  
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JOURNAL: Journal of Molecular Evolution 40 (6):p594-600 1995  
ISSN: 0022-2844  
DOCUMENT TYPE: Article  
RECORD TYPE: Abstract  
LANGUAGE: English

ABSTRACT: Rhodopsinlike opsins constitute a distinct phylogenetic group (Yokoyama 1994, Mol. Biol. Evol. 11:32-39). This **RH2** group includes the green-sensitive opsins in chicken and goldfish and the blue-sensitive **opsin** in a nocturnal lizard gecko. In the present study, we isolated and sequenced the genomic DNA **clones** for the **RH2** **opsin** gene, **rh2**-Ac, of the diurnal lizard Anolis carolinensis. This single-copy gene spans 18.3 kb from start to stop codons, making it the longest **opsin** gene known in vertebrates. Phylogenetic analysis strongly suggests that **rh2**-Ac is more closely related to the chicken green **opsin** gene than to the gecko blue **opsin** gene. This gene tree differs from the organismal tree, where the two lizard species should be most closely related, implying that **rh2**-Ac and the gecko blue-sensitive **opsin** genes have been derived from duplicate ancestral genes.

19/7/4

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06274519 BIOSIS NO.: 000086108702  
ECTOPIC EXPRESSION OF A MINOR DROSOPHILA **OPSIN** IN THE MAJOR  
PHOTORECEPTOR CELL CLASS DISTINGUISHING THE ROLE OF PRIMARY RECEPTOR AND  
CELLULAR CONTEXT  
AUTHOR: ZUKER C S; MISMER D; HARDY R; RUBIN G M  
AUTHOR ADDRESS: DEP. BIOCHEM., UNIV. CALIFORNIA, BERKELEY, CALIF. 94720.  
JOURNAL: CELL 53 (3). 1988. 475-482. 1988  
FULL JOURNAL NAME: Cell

CODEN: CELLE  
RECORD TYPE: Abstract  
LANGUAGE: ENGLISH

ABSTRACT: We have used P-element-mediated transformation to introduce the  
\*\*\*cloned\*\*\* Rh1 rhodopsin gene into the germ line of Drosophila and  
fully rescue the visual phenotype of mutant ninaE flies. A  
transcriptional fusion between the ninaE promoter and the structural gene  
for a minor \*\*\*opsin\*\*\* (\*\*\*Rh2\*\*\*), that is not normally expressed in the  
R1-R6 photoreceptor cells was used to demonstrate that \*\*\*Rh2\*\*\*  
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that specifically affect the R1-R6 cells, ninaA and rdgB, do not directly  
affect expression of the ninaE gene.

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